Morphological Changes in Mouse and Rat Erythrocytes upon Exposure to Cholinesterase Organophosphorus Inhibitors

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Exposure to some bioactive factors is known to induce changes in erythrocyte shape in animals. Bearing in mind that cholinesterase (CE) is present in erythrocytes and that the physiological significance of this enzyme is not yet sufficiently known, we thought it interesting to investigate the morphology of these cells during exposure to CE inhibitors. Heparin-resistant disturbances of blood rheology in Malathion poisoning are mentioned in manuals of clinical toxicology.

MATERIALS AND METHODS

Outbred female white mice and rats were used in the experiments. The organophosphorus compounds phosphacol (paraoxon), synthesized at the Research Institute if Military Medicine, and commercial carbofos (Malathion) in the form of a 47.2% solution were used as CE inhibitors. The mean lethal doses were determined from tables. Blood for investigations was collected after decapitation of the animals. Whole blood CE activity was assessed from the acetylcholine iodide hydrolysis rate (final concentration 1×10^{-2} M) by continuous potentiometric titration with 0.02 N solution of KOH at pH 7.4 and temperature 37°C using a Radiometer autotitrator (Sweden).

Erythrocyte surface relief was analyzed under a scanning electron microscope. Erythrocytes precipitating on a slide were fixed in 1.5% glutaraldehyde with 0.1 M phosphate buffer, pH 7.2, for one hour. After dehydratation in ascending grades of ethanol solutions, the preparations were dried by CO_2 critical point transfer in a HCP-2 device (Hitachi). After dusting with gold with an ionic pulverizer the preparations were examined and photographed using a Hitachi-H-300 scanning electron microscope.

RESULTS

Four series of *in vitro* and *in vivo* experiments were carried out: the first series included studies of rat whole blood CE activity and erythrocyte morphology after intraperitoneal injections of Malathion in doses LD_{50}=439±25 mg/kg; the second series consisted of similar experiments on mice with intramuscular Malathion injections in doses 1/3 and 1/10 LD_{50} (LD_{50}=714±56 mg/kg; the third series was on mice intramuscularly injected phosphacol in doses LD_{50}=0.92±0.12 mg/kg; the fourth series was devoted to studies of erythrocyte morphology after the addition to a washed murine erythrocyte suspension of phosphacol in a final concentration of 0.167 mg/kg providing CE activity inhibition by 92.4% after 15 min incubation with and with-

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TABLE 1. Whole Blood Cholinesterase Activity Inhibition (in Percent vs. the Control) in Mice and Rats in Various Periods after Phosphacol and Malathion Poisoning ($\bar{X} \pm s$)

Agent	Animal	Route of administration	Dose in LD_{50} fractions	Time elapsed after injection of agent						
				minutes				hours		
				5	15	25	30	1	4	24
Malathion	Rats	i/p	LD_{50}	–	100	–	100	100	100	–
	Mice	i/m	1/3	68±8	80±10	93±11	–	–	89±10	–
	Mice	i/m	1/10	45±7	69±6	80±7	–	–	80±10	–
Phosphacol	Mice	i/m	LD_{50}	–	100	–	–	100	100	58±9

Note: each group consisted of 7 animals.

out acetylcholine in final concentration of 1×10^{-4} M, 1×10^{-6} M, and 1×10^{-8} M.

The results of CE activity measurements are presented in Table 1.

In control experiments carried out during all series of investigations the erythrocytes were characterized by a typical normal biconcave disk shape, as shown by scanning electron microscopy (Fig. 1, *a*).

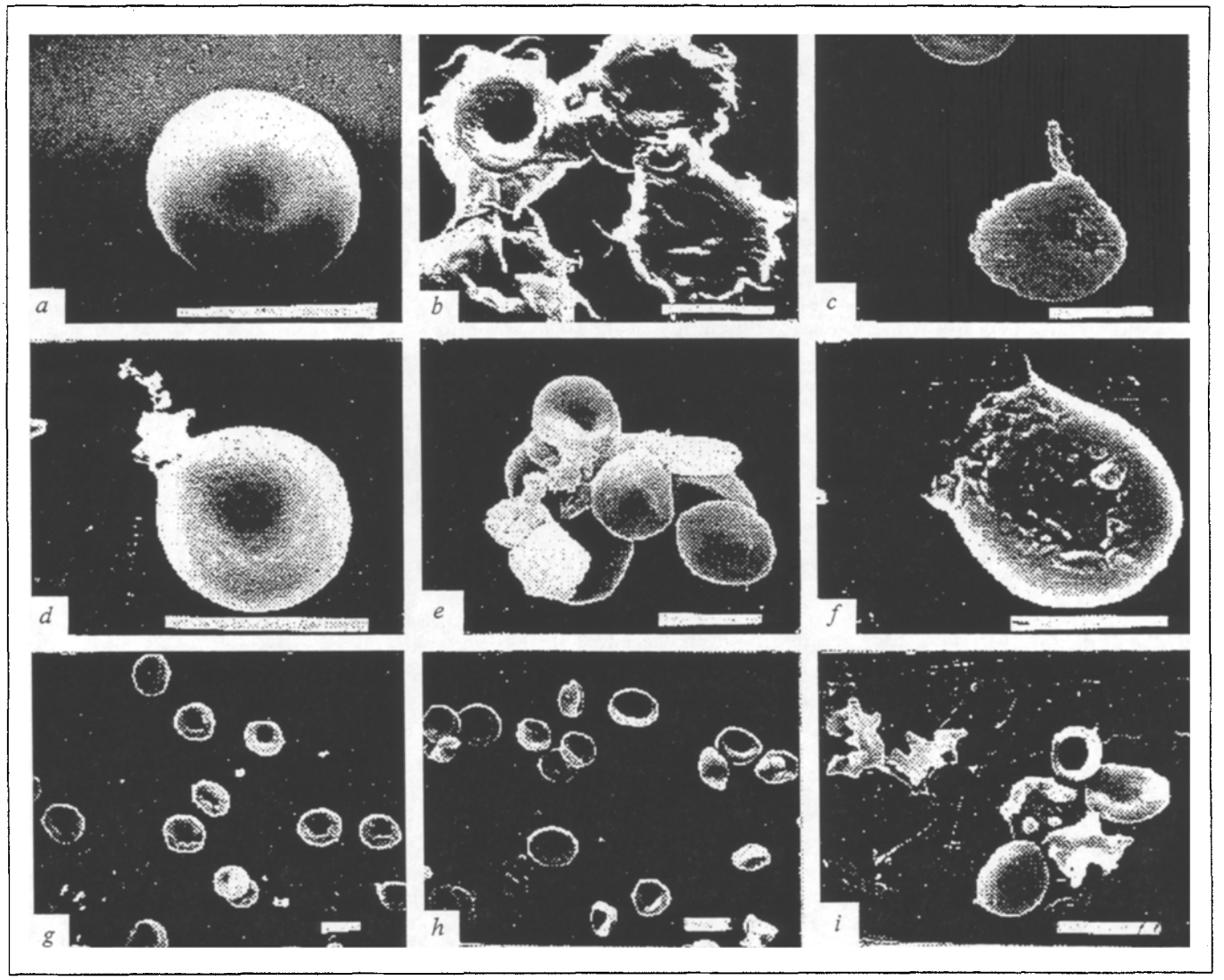

Fig. 1. Scanning electron microscopy of erythrocyte surface (gold dusting). *a*) the norm; *b*) 15 min after intraperitoneal injection of Malathion in LD_{50} dose to rats; *c*) the same after 4 h; *d*) 5 min after intramuscular injection of Malathion 1/3 in LD_{50} dose to mice; *e*) the same after 15 min; *f*) 15 min after intramuscular injection of phosphacol in LD_{50} dose to mice; *g*) the same after 4 h; *h*) 30 min after *in vitro* incubation of rat blood with phosphacol in a concentration of 0.167 mg/ml; *i*) the same 15 min after the addition of acetylcholine in a concentration of 1×10^{-8} M to the medium. Scale: 5μ

The preservation of the characteristic shape and size (5.5±0.7 μM, $\bar{X}\pm s$) of erythrocytes in control tests and the minimal number of deformed elements indicate the adequacy of the methods used for preparation of the material for investigation.

Malathion in the LD_{50} dose significantly changed erythrocyte size and shape. Swelling with characteristic changes of shape and the development of plasmalemma defects were observed 15 min after Malathion administration to rats. The formation of erythrocyte aggregates (Fig. 1, *b*) was a characteristic reaction. No drastic changes in erythrocytes were seen after 4 h. Among the residual phenomena were excrescences on the cell surface (Fig. 1, *c*).

Injections of lower Malathion doses (1/3 and 1/10 LD_{50}) to mice were associated with similar changes of cell surface: swelling, plasmalemma defects (Fig. 1, *d*). Note that the recovery of normal erythrocyte morphology depended to a great extent on the dose of organophosphorus inhibitor. Deformed erythrocytes were detected 25 min after injection of 1/3 LD_{50} of Malathion, whereas when 1/10 LD_{50} was injected, deformed erythrocytes disappeared from the blood at he 15th min after injection of the insecticide.

Significant changes in erythrocyte surface relief are observed in the animals' blood after phosphacol injection in the LD_{50} dose. The size of the cells increases to 8.3±1.2 μ ($p<0.05$). The erythrocytes swell and lose the biconcave disk shape. Many cells develop plasmalemma defects associated with erythrocyte destruction (Fig. 1, *e*). Numerous excrescences are seen on the surface, making the cells look like echinocytes. One hour after phosphacol injection in a sublethal dose the cell surface normalized and cell size decreased to 4.3±0.8 μ ($p=0.25$). The pathological shifts were still less manifest 4 h after phosphacol injection: cell size almost recovers (5.1±0.5 μ), and the cells reacquire the characteristic shape of a biconcave disk (Fig. 1, *f*).

The virtually complete recovery of the morphology of formed elements of the blood under conditions of persistent suppression of blood CE activity in 90% of experiments gives grounds to assume that erythrocyte deformation is due to appearance of an acetylcholine "wave" in the blood emerging almost immediately after administration of an organophosphorus inhibitor of CE but disappearing with the adaptation of the cholinergic synapses.

For verification of the hypothesis about the deforming action of acetylcholine an almost complete inhibition of CE activity was attained *in vitro* by the addition of phosphacol in a final concentration of 0.167 mg/ml. Thirty minutes after exposure, 60% of erythrocytes were reduced in size to 4.0±0.5 μ and the cell surface was slightly deformed (Fig. 1, *g*). The addition of acetylcholine to the medium (Fig. 1, *h*) during blood fixation induced within 15 min the appearance of numerous deformed erythrocytes; their number as growing the transmitter concentration increased from 1×10^{-8} to 1×10^{-4} M. We may assume the altered cell surface relief to be related to an active responses of supporting-contractile (cytoskeletal) structures. Our data demonstrate that erythrocyte deformation may be explained, among other things, by the effects of acetylcholine accumulating in the blood during poisoning with organophosphorus CE inhibitors or of acetylcholine added to the erythrocyte suspension under conditions of preliminary suppression of its hydrolytic activity. Hence, one may speak of the protective role of CE, which is able to mitigate the pathological effect of acetylcholine.